In vitro digestibility and quality analysis of enzymatic protein extracts generated independently from the red seaweed *Chondrus crispus*, buckwheat and spelt grains.

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INTRODUCTION

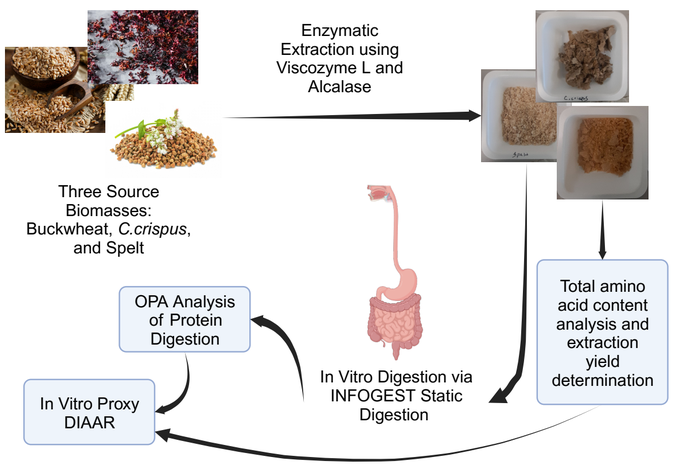
Interest in novel plant-based proteins is increasing as a larger percentage of many populations are choosing to reduce or replace traditional animal-based protein sources within their diets. A major limitation of plant-based protein concerns low amino acid digestibility compared to dairy and meat. Plant proteins often have low concentrations of essential amino acids like lysine and the sulphur amino acids.

Protein extracts were generated from the red seaweed *Chondrus crispus*, a red seaweed rich in protein.

Extracts from buckwheat and spelt were also generated to provide a land-based comparison from amino acid rich grains.

Multiple protein extraction methods were tested, with enzymatic hydrolysis providing the highest protein extraction yield and amino acid profiles that best met dietary requirements.

METHODS



Protein extracts were generated via mixing biomass in water at a 20:1 w:v ratio, incubating with 3% w:v Viscozyme L for 3 hours then for three hours with 3% w:v Alcalase. Following this the soluble phase was collected and freeze dried to create the enzymatic protein extract.

Enzyme Extracts were analysed for total amino acid content. This measurement was then used to calculate amino acid nitrogen which was used to determine protein content using a protein to nitrogen conversion factor of 6.25.

In vitro digestion of protein extracts was performed in accordance to the INFOGEST method.

O-phthalaldehyde (OPA) analysis of protein digestion and proxy in vitro DIAAR determination was performed based on the methodology out lined in *Sousa et al 2023*

CONCLUSION

Enzyme extraction resulted in a significant increase in essential amino acids content for buckwheat and spelt (30.79mg/g to 38.01mg/g & 28.90mg/g & 38.10mg/g)

All protein extracts are highly digestable with digestibility exceeding 80%

In vitro proxy DIAAR values that exceed 75% are considered to be a good amino acid source. Based on this analysis both buckwheat and spelt extracts could be considered good sources of dietary amino acids. *C.crispus* extract is limited by the DIAAR of histidine

DIAAR values greater 100% indicate an amino acid source that exceeds dietary requirements

RESULTS

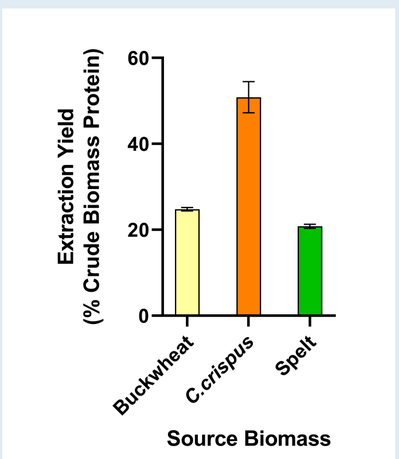
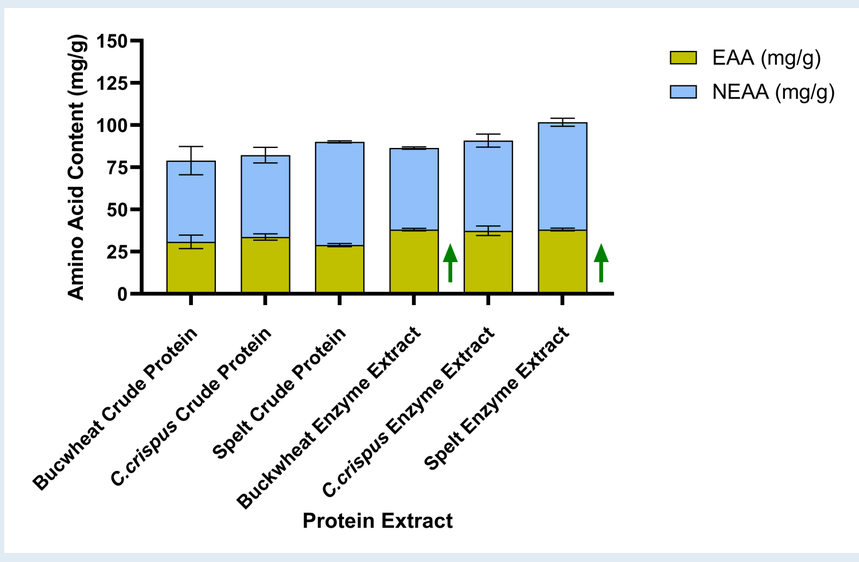


Figure1. Protein Extraction Yield of Enzymatic Protein Extracts.
Mean dry matter protein yield in percentage of crude biomass protein± SD. Samples of each protein extract where testing using the amino acid nitrogen (AAN) method.

Figure2 . Protein Amino Acid Composition in Crude Biomass and Protein Extract.
Data is represented as mean ± standard deviation of essential amino acids (EAA) and non essential amino acid (NEAA) content. Amino acid content is measured in milligrams per gram of dry matter sample, either crude protein or enzyme extract. Green arrows indicate a statistically significant increase in extract amino acid content compared to the crude protein of the same biomass (P<0.05)



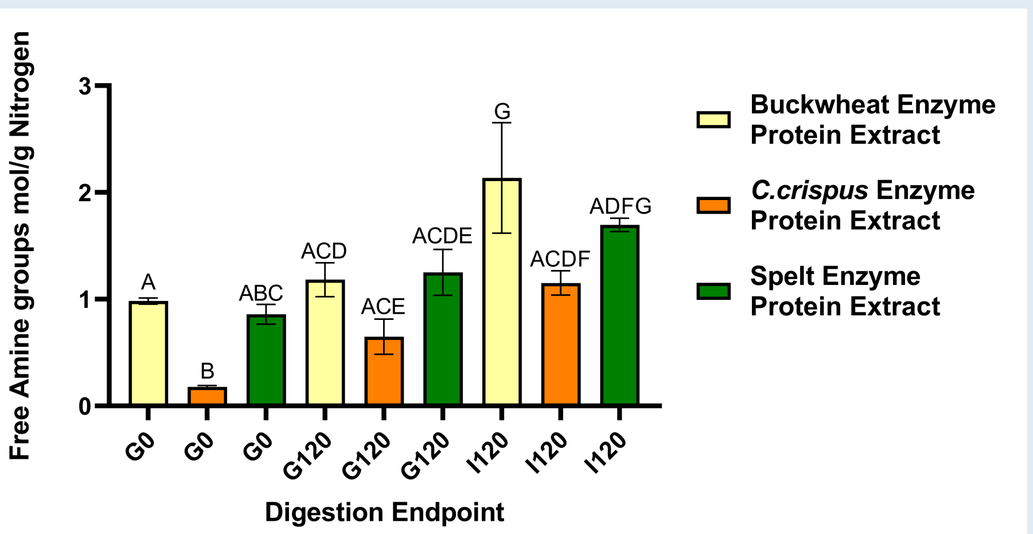


Figure 3. OPA Analysis of Protein Digestion.
Concentration of free amine groups per gram of sample nitrogen (G0), gastric end phase (G120) and intestinal end phase (I120). Samples were generated using INFOGEST digestion method. Data represents mean ± Standard deviation, n = 3, matching letters indicate no significant difference.

Table 1. Digestibility of Enzyme Extract Protein.
This table represents overall protein extract digestibility based on OPA analysis. Data is represented as mean ± standard deviation.
Overall digestibility was determined by comparing the OPA value of the bioavailable fraction as a percentage of the total sample of protein extracts at the end of the intestinal digestion phase.

Enzyme Extract	Digestibility (%)
Buckwheat	80.7 ± 7.5(%)
C.crispus	81.0 ± 8.9 (%)
Spelt	89.6 ± 2.4(%)

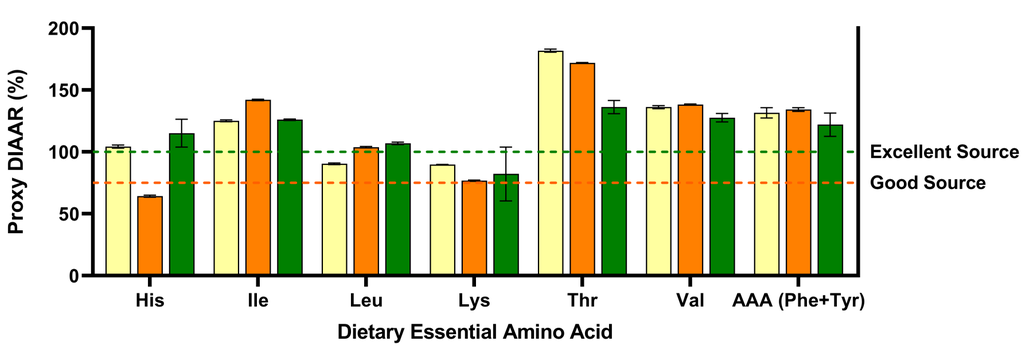


Figure 4. In vitro Proxy DIAAR.
Data is represented as mean ± standard deviation, n = 3. In vitro proxy DIAAR is calculated based on initial analysis of amino acid content of enzymatic protein extracts multiplied by the extract digestibilities shown in **Table1.** To calculate the in vitro proxy DIAA. The calculated proxy DIAA was then compared against the FAO dietary requirement scoring pattern for 6 months to 3 years old to calculate the in vitro proxy DIAAR. DIAAR represents how well the amino acid content of an extract satisfy dietary requirements.

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Acknowledgements:
Ethan Cain is Currently in receipt of a Teagasc Walsh Scholarship (2018049). This work is funded by Teagasc under project MBDY0360 BIOPROTEIN

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